

REMARKS

The amendments set out above and the following remarks are believed responsive to the points raised by the Office Action dated September 7, 2004. In view of the amendments set out above and the following remarks, reconsideration is respectfully requested.

The Present Invention

The present invention relates to a method of culturing a microorganism for the synthesis of docosahexaenoic acid (DHA) by the microorganism, comprising culturing *Cryptocodinium cohnii* with a compound selected from acetic acid and an acetate ion, the microorganism using the compound as the primary carbon source and synthesizing DHA in the absence of a stationary phase.

The Pending Claims

Claims 58, 59, 65, 66, and 83-86 have been cancelled by this amendment, and claims 34, 37-51, 74-79, 81 and 82 remain pending. Claim 34 is the sole independent claim. Claims 34, 40, and 41 have been amended to describe the invention more clearly. No new matter has been added, the basis for the amended claim language may be found within the original specification, claims and drawings.

The Office Action

For convenience, the following remarks will address the various comments and rejections in the same order they were raised in the Office Action.

The Office Action indicated that the claims drawn to a non-elected invention should be cancelled. Accordingly, claims 58, 59, 65, 66, and 83-86 have been cancelled without prejudice to re-file these claims in one or more continuing applications.

The Office Action indicated the previous response contained an amendment failing to comply with the Revised Amendment Format 37 CFR 1.121, at least claim 40 allegedly being amended without the appropriate indication of brackets, strike-throughs and/or underlining. Applicants have thoroughly reviewed the previous amendment, including the amendment to claim 40, and have not identified any amendments failing to comply with the Revised Amendment Format. Accordingly, it is respectfully submitted the objection should be withdrawn.

The Office Action asserts that for the sake of clarity and correctness, the limitation, "in a medium" should be added to claim 34. Although Applicants believe the claim is clear and

correct as written, in order to expedite matters, the limitation, "in a medium" has been added to claim 34.

Claims 34, 36-51, and 74-82 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 34, 36-51, and 74-82 were also rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Office Action alleges that no basis or support is found in the present specification for "comprising culturing a microorganism comprising *Cryptocodinium cohnii*" and that claim 34 is vague and indefinite. Claim 34 has been amended to refer to "culturing *Cryptocodinium cohnii*". The Office Action also alleges that claim 34 remains confusing and vague in that it is "unclear how the absence of a stationary phase can be successfully determined, since all is requires for growth to stop."

Applicants respectfully submit that one of skill in the art could readily determine that a microorganism is being cultured in the absence of a stationary phase. Those of skill in the art can readily determine that a stationary phase is induced when culturing a microorganism, for example as illustrated by one of the cited references (U.S. Patent No. 5,407,957 at col. 5, lines 23-27) where a stationary phase is induced by providing YE (yeast extract) in a limiting amount such that the medium runs out of YE while available glucose remains. Conversely, one of skill in the art would recognize that a microorganism is being cultured in the absence of a stationary phase when conditions creating a stationary phase are not imposed.

The Office Action also alleges that no basis or support is found in the specification for "the use of a means to monitor pH as a 'controller'" and that claim 41 is vague and indefinite. Claims 41 and 42 have been amended to replace the term "controller" with "control device", the specific term used in the specification, for example, at page 5, paragraph 2, and page 8, lines 7-12. Claim 40 is directed to a method where the pH is monitored by means communicating with a control device, the control device controlling the addition to the medium. The specification clearly explains the operation of the control device (at page 5, paragraph 2 and referencing Figure 1) stating, "The control device 12 is programmed to cause the pump 13 to pump a liquid from a reservoir 14 to the fermenter 10 when the pH electrode 11 detects a pH greater than a predetermined value." Thus, the control device communicates with means for monitoring the pH (e.g., a pH controller) and in response to signals from the means for monitoring the pH, controls addition of the compound to the medium.

Thus, it is respectfully submitted that with these remarks and amendments to the claims, the bases for rejection under 35 U.S.C. §112 have now been overcome and should be withdrawn.

Claims 34, 36-51, and 74-82 were rejected under 35 U.S.C. §103(a) as being unpatentable over Vazhappilly et al. (hereinafter referred to as "Vazhappilly") in view of U.S. Patent No. 5,407,957 to Kyle et al. (hereinafter referred to as "Kyle") and Du Preez et al. (hereinafter referred to as "Du Preez"). This rejection is respectfully traversed.

The present invention is directed to a method of culturing a microorganism comprising culturing *Cryptocodinium cohnii* to synthesize docosahexaenoic acid (DHA) in the absence of a stationary phase. The imposition of a stationary phase, as is known to those skilled in the art, may comprise creating a nutrient deficiency, e.g., a nitrogen deficiency, in the culture which induces the production of DHA in the microorganism (see e.g., Kyle and U.S. Patent No. 5,711,983 to Kyle et al.). Prior to the present invention, and as evidenced by the absence of any teaching in the cited references, it was not known that DHA could be synthesized without the imposition of a stationary phase. For example, as explained in Kyle,

"Production of the single cell oil is induced in the dinoflagellates by the imposition of a nitrogen deficiency. Such deficiencies are caused by providing YE in a limiting amount such that the medium runs out of YE while available glucose remains."

Indeed, Kyle specifically states, "The present invention recognizes that it is the carbon source to nitrogen source ratio which promotes the efficient production of the single cell oil". Thus, Kyle explicitly teaches that it is the imposition of the stationary phase that promotes the production of the oil.

The Office Action correctly notes that the main thrust of Applicants argument is that the Kyle reference requires the imposition of a stationary phase for the production of DHA. However, the Office Action goes on to state that an "induction" step cannot be considered part of the "culturing" process for the production of DHA *per se*, but rather constitutes one or more pre-culture process steps and states that this distinction is clearly apparent at least from Example 2 where the cells are pre-cultured in acetic acid/acetate. Applicants respectfully disagree.

Pre-culturing a microorganism, i.e., preparing the inoculum, used in a culturing method is very different from inducing a stationary phase during the subsequent culturing of the microorganism. As explained in the present specification, a concentration of microorganisms to be used in the culture, i.e., "the inoculum", is prepared (see e.g., page 6, paragraph 2) prior to initiating the culture of the microorganism. Regardless of the way in which the inoculum is prepared, in accordance with the present invention, the subsequent culturing is carried out in the absence of a stationary phase. For example, while the method of preparing the inoculum is

different in Examples 1 and 2 of the present application, the subsequent culture of the prepared inoculum is the same and is in the absence of a stationary phase (see e.g., page 15, lines 10-14).

In the prior art, the stationary phase is induced during the culturing of the inoculum, i.e., subsequent to the preparation of the inoculum. For example, Kyle (col. 7, lines 1-34) teaches that *inoculum* from a seed fermenter is added to another fermenter with glucose and a limiting amount of YE (i.e., to create a nitrogen deficiency and induce a stationary phase), and the culture is permitted to grow and the oil produced by the microorganisms is harvested. Thus, Kyle makes clear that it is while *culturing* the inoculum that the stationary phase is imposed. In contrast, in the present invention the microorganism is cultured in the absence of a stationary phase.

Clearly, there is a distinction between the pre-culture steps used to prepare the inoculum and the induction step to induce a stationary phase while culturing the inoculum. The presently claimed invention defined in independent claim 34 is directed to a method including culturing *Cryptocodinium cohnii* in the absence of a stationary phase, and neither Kyle nor any of the other cited references discloses such a method.


For the reasons set forth above, reconsideration of the rejection is respectfully requested.

Conclusion

In view of the amendment and remarks recited herein, the application is considered in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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